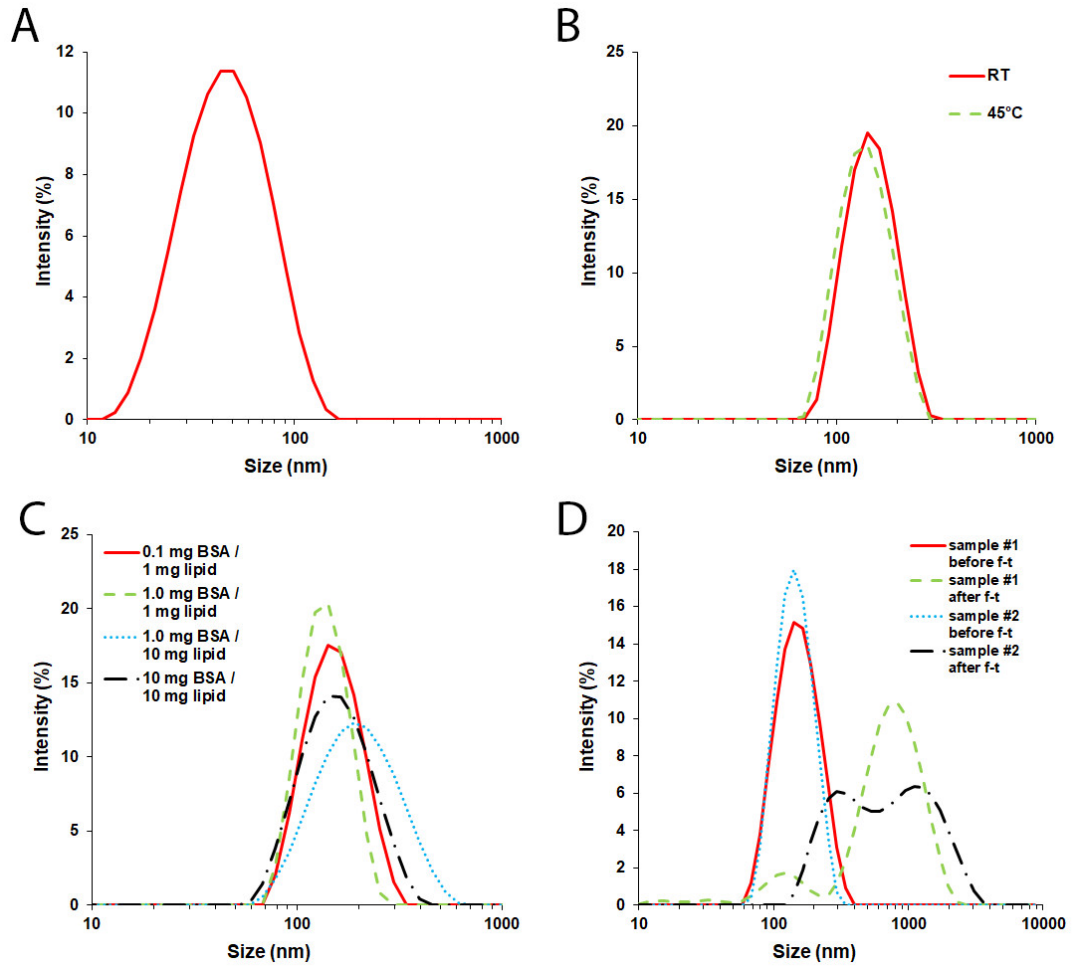
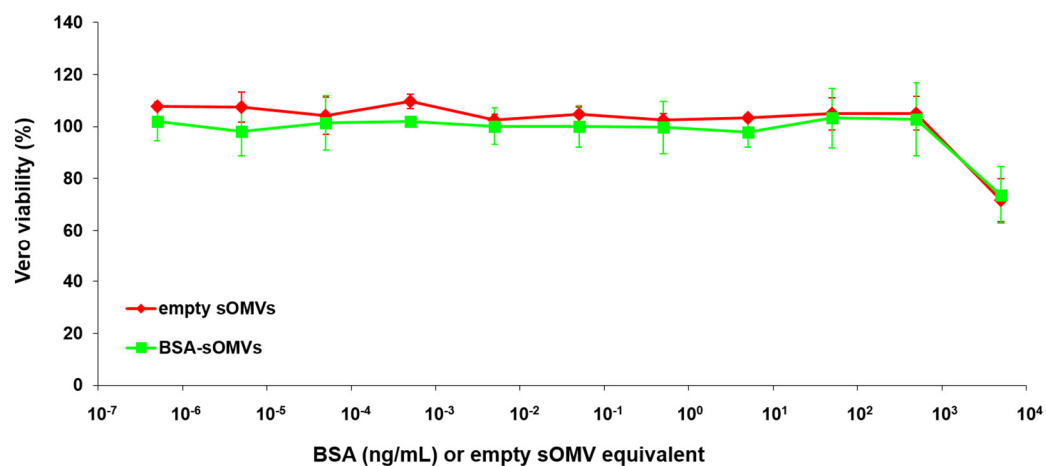


# Supplementary Materials



**Figure S1.** Parameters evaluated for sOMV preparation. Depicted is the size distribution of liposomes (A) and sOMVs (B-D) as measured by DLS. (A) POPG/POPE/CL liposomes after extrusion and before the addition of protein. (B) BSA-loaded sOMVs after ultracentrifugation with the complete preparation procedure performed at 45 °C or changed to RT upon addition of protein. (C) BSA-loaded sOMV preparations with different ratios of protein and lipid, as indicated. (D) BSA-loaded sOMV preparations initially stored at 4 °C before and after freezing with subsequent thawing (freeze-thawing, f-t) either conventionally frozen in the freezer at -20 °C (#1) or deep-frozen in liquid nitrogen at -196 °C (#2).



**Figure S2.** Effect on cell viability of lipids used in sOMV preparation and cargo in general. Cytotoxicity of empty sOMVs without cargo and BSA-loaded sOMVs toward Vero cells, as determined by the crystal violet assay. For empty sOMVs, an amount equivalent to the one of BSA-loaded sOMVs was applied. Depicted is the mean  $\pm$  standard deviation in relation to an untreated control of three biological replicates ( $n = 3$ ) each performed in triplicates.